The influence of surface patterning on bacterial growth behavior

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The interplay between surface properties and the growth behavior of bacteria on these surfaces is of significant interest for the understanding of microbial colonization of devices, such as bioreactors or medical implants and instruments. While lithographic processes for defining surface structures are well-established in the nano-fabrication community, these processes are just beginning to find their way into other academic disciplines, e.g. microbiology[1]. Until now, experiments addressing the influence of physical surface properties on bacterial growth have mainly focused on surface structures obtained by common industrial fabrication processes like wet chemical etching or (sand) blasting[2]. These processes are capable of achieving nanometer-sized surface features over large surface areas, but with rather randomly distributed microgeometries. Therefore, they do not provide the experimentalist with the means to tune geometric parameters to the same degree of freedom as is possible with lithographic methods.

In our approach, we take advantage of lithographic processes to fabricate templates for the replication of poly(dimethyl)siloxane (PDMS) substrates used in microbiological growth studies (see figure 1). The different surface patterns that are obtained in this manner lead to a set of environments with distinct microgeometries for bacterial growth on a single substrate, therefore having identical surface chemistry. The influence of the different surface structures is then investigated by growing bacteria, in this case *Staphylococcus aureus*, on the substrates and observing the resulting growth patterns exhibited by the bacterial colonies by fluorescence microscopy and scanning electron microscopy.

Our findings suggest that, as far as *S. aureus* is concerned, bacterial growth patterns are greatly influenced by the underlying surface geometry (see figure 2). The bacterial colonies react to surface features on a broad range of geometric parameters, ranging from a few hundred nanometers to several micrometers. Correlations between geometrical parameters and growth patterns will be elaborated.

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[1] D. B. Weibel et. al., Nat. Rev. Microbiol. , **5**, 209 (2007)
[2] X. Wang et. al., Surf. Coat. Tech., **203**, 3454 (2009)

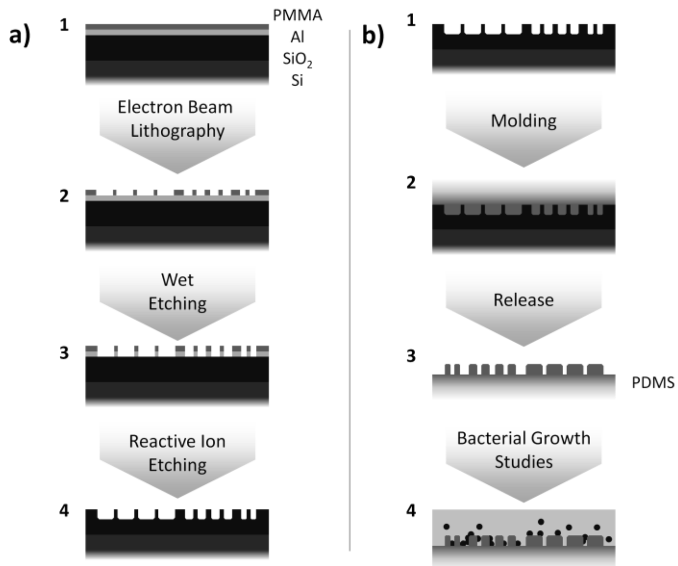

Figure 1 - Schematic fabrication and handling processes

a) *Template fabrication:* Starting from a material stack with polymethyl-methacrylate (PMMA), aluminum (Al), silicon dioxide (SiO_2) on silicon (Si) (step 1), geometric patterns are created lithographically (step 2) and etched into the Al layer (step 3) and finally into the SiO_2 layer (step 4).

b) *Substrate fabrication:* The template is immersed in the liquid PDMS precursor (steps 1 & 2). After cross-linking of the PDMS, the substrate is released (step 3) and ready for bacterial growth studies (step 4).

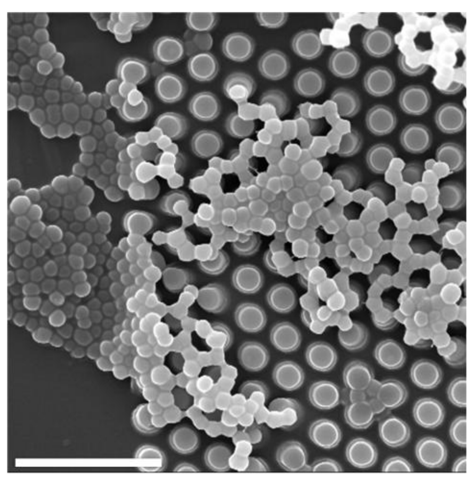

Figure 2 - Influence of substrate structure on bacterial growth patterns

The scanning electron microscopy image shows Staphylococci on a substrate with areas of hexagonally arranged PDMS pillars. Note the different bacterial growth behaviors as the surface topography changes. (Scale bar: 10µm)